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BrucellaCapt versus Classical Tests in the Serological Diagnosis and Management of Human Brucellosis▽

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The BrucellaCapt test is an immunocapture agglutination test suggested as a possible substitute for the Coombs test in the diagnosis of human brucellosis. Here it is compared with classical tests using 321 samples from 48 patients with brucellosis (6.9 ± 1.7 samples per patient), including 20 patients with focal disease and 8 patients with a total of 9 relapse episodes (mean follow-up, 18 months). The BrucellaCapt test was used according to the manufacturer's instructions, and we also used a variant of the BrucellaCapt test in which the microtiter plates were not coated with antibodies against total human immunoglobulin (BCAPV). The correlation between the BrucellaCapt and BCAPV tests was 0.982 ($P < 0.001$), with 260 coincident pairs of titers (81%). The areas under the receiver operating characteristic curve for the BrucellaCapt and BCAPV tests with respect to the Coombs test were 0.969 and 0.960, respectively. Upon admission, the BrucellaCapt, BCAPV, and Coombs tests and the microagglutination test (MAT) were positive for all cases: titers were 1/2,560 by the BrucellaCapt test, 1/2,560 by the BCAPV test, 1/1,280 by the Coombs test, and 1/320 by the MAT. The decreases in the BrucellaCapt and BCAPV titers over time were pronounced in comparison with the Coombs titers. Cumulative probabilities of persistence 12 months after therapy were as follows: 80% by the BrucellaCapt test, 80% by the BCAPV test, 87% by the Coombs test, and 35% by the MAT. Serological changes during relapse were detected in seven cases (88%) by the Coombs test, in five cases by the BrucellaCapt and BCAPV tests, and in three cases by the MAT. The BrucellaCapt test is a sensitive, specific, and simple test for routine use in human brucellosis. Similar results were obtained with the BCAPV test. However, in some cases of relapse and chronic forms of the disease, the slight changes observed in low-affinity antibodies alone are better detected by the Coombs test.

Human brucellosis continues to be a major health problem worldwide. Although the endemicity of this disease is limited to some areas of the Mediterranean basin and developing countries in Asia, Africa, and Latin America, sporadic cases may develop in any area where the disease is not endemic. Thus, the illness is currently included among travelers' diseases (20).

The definite diagnosis of the disease is based on the isolation of *Brucella* spp. in blood cultures (12, 32), but under certain clinical conditions the microorganism cannot be finally recovered. In the past decade, PCR has emerged as a promising alternative method to confirm the presence of the microorganism (16, 23), although its use has not been standardized. Thus, serological tests continue to play a relevant role in the diagnosis and management of patients with brucellosis (12, 31). Most classical tests used, along with the enzyme-linked immunosorbent assay method, offer good detection of anti-lipopolysaccharide agglutinating and/or nonagglutinating antibodies. However, problems in serological diagnosis continue, mainly in chronic forms of the disease and in the course of follow-up, when the meaning of persistent titers could be difficult to interpret, especially since no definite criteria of cure have yet been established (2, 12, 13, 18, 24, 25).

In recent years, the new immunocapture agglutination anti-*Brucella* (BrucellaCapt [BCAP]) test has been reported to detect agglutinating and nonagglutinating antibodies with very high sensitivity (1, 5, 6, 8, 10, 15, 17, 18, 27, 29). It has been suggested as a possible substitute for the anti-human immunoglobulin (Coombs) test and, perhaps, as a better marker of disease activity (1, 17, 27, 29). The aim of the present study was to investigate the basis and mechanisms of the high sensitivity of the BCAP test and to conduct an accurate evaluation of its performance in comparison with those of classical tests for a large group of brucellosis patients with several forms of the disease over a prolonged follow-up period.

MATERIALS AND METHODS

Patients and serum samples. Serum samples from 48 patients diagnosed with brucellosis at Bellvitge Hospital, a tertiary teaching hospital in Barcelona, Spain, were included. These patients were part of a series of patients with brucellosis who were treated and prospectively followed up in our hospital over a period of years, as reported elsewhere (2, 3). For all patients with positive blood cultures, *Brucella melitensis* was identified. The criteria used to select the 48 cases were (i) the availability of frozen serum samples; (ii) complicated brucellosis, focal disease, or relapse; and (iii) prolonged follow-up. The diagnosis of brucellosis was based on clinical findings and the positivity of blood cultures for *Brucella* or a microagglutination test (MAT) titer of ≥1/160. Routine blood cultures and serological and clinical evaluations were performed upon admission, at the end of treatment, and at the 1st, 2nd, 3rd, 6th, 9th, 12th, and 18th months after therapy. In cases with clinical relapse, an additional sample was obtained for bacteriological and serological studies. Twenty-milliliter blood samples were

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frozen at −30°C until processing for specific serological studies. Relapse was defined as the reappearance of signs or symptoms of the disease and/or a positive blood culture after therapy. Focal disease was defined as the persistence of signs or symptoms of infection at a particular anatomic site for >7 days.

Serological methods. Serological studies were carried out in the microbiology laboratories of the Bellvitge Hospital and the Clinica Universitaria de Navarra. The Rose Bengal (RB) test, the MAT, the Coombs test in microtiter plates, the BCAP test, and a variant of the BCAP test in which microtiter plates were not coated with antibodies against total human immunoglobulin (the BCAPV test) were performed for each sample. All samples from the same patient were processed simultaneously. Patients whose serum antibody titers upon admission or at 12 to 18 months after therapy were two or more times higher than the median titers for the whole group of patients at these periods were arbitrarily considered to have high initial or high final titers, respectively. For analysis of the probability of persistent significant positivity, titers of <1/40 for the MAT and <1/160 for the Coombs, BCAP, and BCAPV tests were considered negative.

The RB test was performed according to standard procedures (12) using antigen supplied by the Laboratorio Estatal de Sanidad Animal, Santa Fé, Granada, Spain. The MAT was performed on microtiter plates by a double-dilution method from an initial 1/20 dilution of the serum sample with phosphate–buffered saline (PBS) (pH 7.2) and by using the milk ring test antigen (*Brucella abortus*; Central Veterinary Laboratory, Weybridge, Surrey, United Kingdom) diluted 1/70 in PBS (7, 9, 12, 14). The plates were incubated for 18 to 24 h at 37°C. The Coombs test was performed by microtitration according to the method described by Otero et al. (19), with some modifications (15, 26, 28), using anti-human immunoglobulin G (IgG) (Operon, Saragossa, Spain) diluted 1:1,000 in PBS. The BCAP test was carried out according to the manufacturer's instructions (Vircell SL, Santa Fé, Granada, Spain). The test is a single-step immunocapture-agglutination assay and consists of microtiter plates coated with antibodies against human immunoglobulins (IgG and IgA). After the addition and dilution of serum in an acid buffer, the antigen suspension included in the BrucellaCapt kit (colored *B. abortus* bacteria killed by formaldehyde treatment) was added, and the strips were sealed and incubated at 37°C for 24 h in a dark, humid chamber. Positive reactions show agglutination over the bottom of the well. Negative reactions are indicated by a pellet at the center of the bottom of the well. The BCAPV test (a variant of the BCAP test) was performed in an identical way and with the same cell antigen suspension but used microtiter plates that were not coated with the anti-human immunoglobulins included in the BrucellaCapt kit.

Statistical analysis. All data were analyzed by SPSS, version 12.0 for Windows, except those on Bland-Altman graphs, which were analyzed by MedCalc. The Bland-Altman data were calculated using the method of differences. In this graphical method, the differences between the two techniques are plotted against the averages of the two techniques. Horizontal lines are drawn at the mean difference and at the limits of agreement, which are defined as the mean difference ± 1.96 times the standard deviation of the differences. The plot was used to inspect whether the difference and its variance were constant as a function of the average. This was achieved by the correlation of the difference versus the average; a value near zero implied concordance. Serological data were expressed as the median and range of reciprocal titers. Sensitivity, specificity, and likelihood ratios (LR) for positive and negative results were calculated. LR of >10 for positive results and <0.1 for negative results were considered conclusive. The kappa statistic was calculated for assessment of agreement between data titers as categorical ratings. Spearman's correlation coefficient was calculated for the analysis of correlation between titers obtained with the BCAP test, the BCAPV test, the MAT, and the Coombs test. These correlations were separately evaluated for samples from three periods: (i) admission to the first month posttherapy, (ii) the second to sixth month posttherapy, and (iii) thereafter. The sensitivity and specificity results obtained with BCAP and BCAPV titers versus a gold-standard titer obtained by the Coombs test (≥1/160) or the MAT (≥1/80) for different cutoff points were represented by a curve of diagnostic efficiency (receiver operating characteristic [ROC] curve). The area under the curve (AUC) was calculated with a corresponding 95% confidence interval (95% CI). Survival curves were constructed by the Kaplan-Meier method and were compared by using the log rank test. A P value of <0.05 was considered to be statistically significant.

RESULTS

Characteristics of patients. There were 48 patients, 37 men and 11 women (mean age, 40.83 ± 15.7 years; range, 15 to 74 years). Thirty-seven (77%) had blood cultures positive for *Brucella melitensis*. For six patients (shepherds and abattoir workers), the disease was work related. The duration of the disease at diagnosis was 60.3 ± 81.8 days (range, 3 to 365 days). For 20 patients (41.7%), a focal disease was diagnosed: there were eight cases of orchitis (21.6% of male patients), six of sacroiliitis (12.5%), five of spondylitis (10.4%), two of arthritis (4.2%), two of prostatitis (5.4%), and one of neurobrucellosis (2.1%). There was a relationship between the frequency of focal disease and the time of the disease: 10/36 (27.8%) patients with durations of disease of ≤60 days at diagnosis and 10/12 (83.3%) patients with durations of >60 days ($P < 0.05$) had focal disease. Orchitis and sacroiliitis were the most prevalent focal diseases observed for patients with times of evolution of ≤15 days.

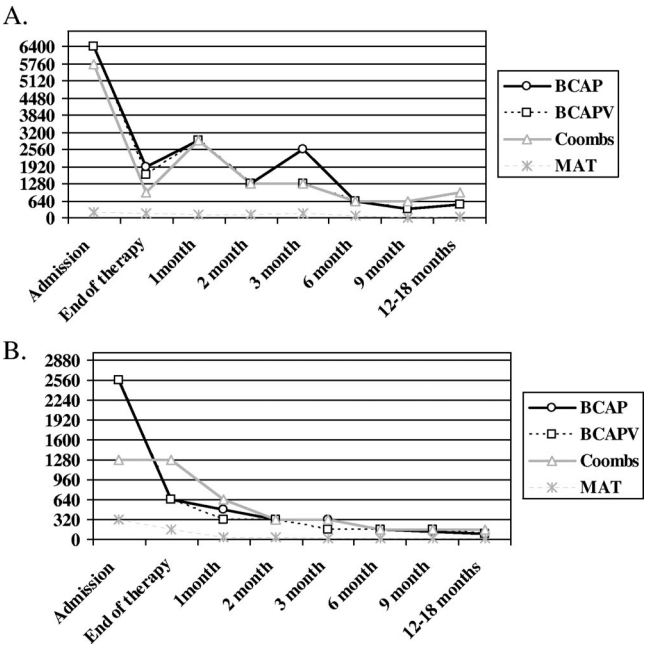

FIG. 1. Serological titers by the MAT and the BCAP, BCAPV, and Coombs tests over time for patients with relapse (A) and without relapses (B). The BCAP line is often hidden behind the BCAPV line.

Three hundred twenty-one samples were studied serologically (3 to 10 samples per patient; mean ± standard deviation, 6.9 ± 1.7 samples). Patients were followed up for a mean period of 18 months (range, 3 to 36 months); 39 patients had a follow-up period of ≥12 months.

Eight patients relapsed after treatment (16.6%), and one of them presented two episodes. The relapse occurred in the 1st month in four cases and in the 3rd month in two cases, and there was one episode each in months 6, 7, and 17. Blood cultures were positive for *B. melitensis* at the time of relapse in four of these nine episodes.

Serological titers over time for patients with and without relapses are shown in Fig. 1A and B, respectively. In the group with relapses, the titers by the BCAP, BCAPV, and Coombs tests decreased more slowly and showed several peaks during this follow-up period. Similar results were observed for MAT titers, but to a lesser extent.

Initial serum antibody titers. All patients underwent initial serological studies either upon admission, during treatment, or

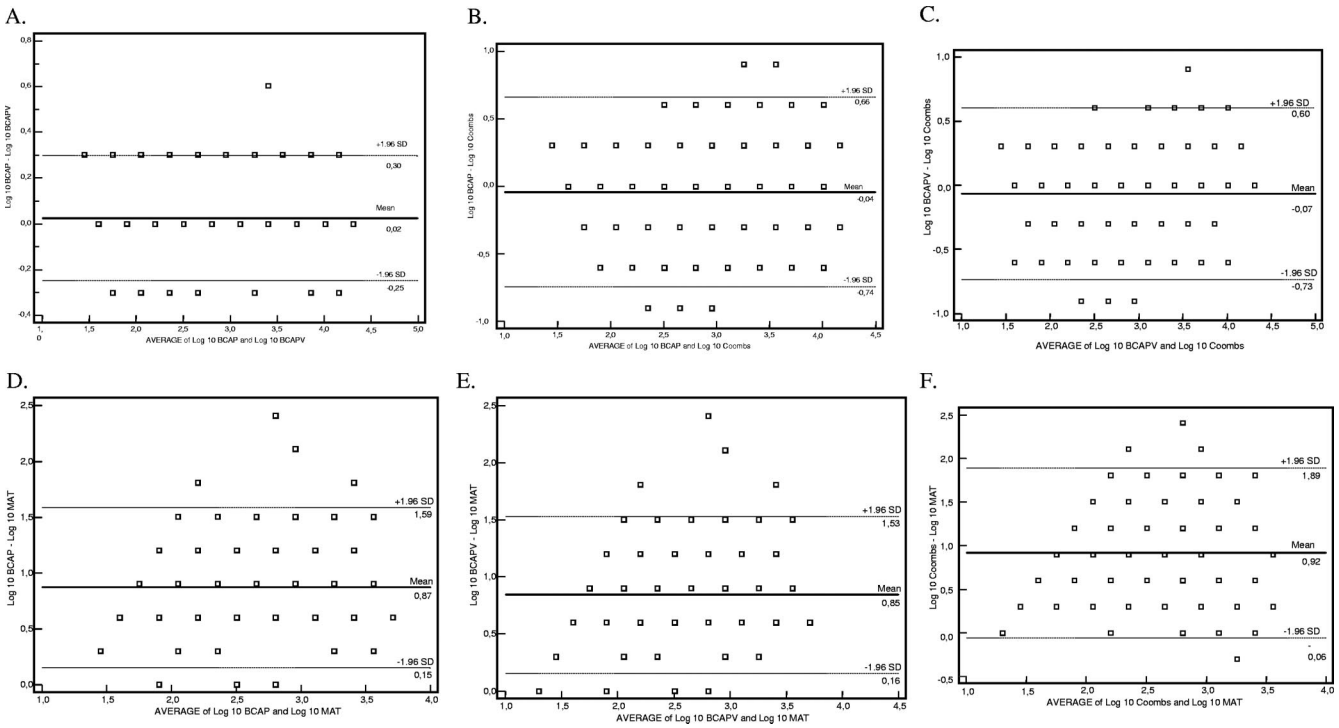

FIG. 2. Bland-Altman graphs showing the levels of concordance of the BCAP, BCAPV, Coombs, and MAT titers. Relationships between the BCAP and BCAPV tests (A), the BCAP and Coombs tests (B), the BCAPV and Coombs tests (C), the BCAP test and the MAT (D), the BCAPV test and the MAT (E), and the Coombs test and the MAT (F) are shown.

at the end of treatment. The median serum antibody titers for 35 patients upon admission were as follows: 1/2,560 (range, 1/320 to 1/81,920) by the BCAP test, 1/2,560 (range, 1/320 to 1/81,920) by the BCAPV test, 1/1,280 (range, 1/160 to 1/20,480) by the Coombs test, and 1/320 (range, 1/40 to 1/20,480) by the MAT. The RB test, the MAT, and the Coombs, BCAP, and BCAPV tests were positive in all cases, but for two patients with blood cultures positive for *B. melitensis* and with a disease duration of 90 days before admission, the MAT titer was 1/40.

Eighteen patients had high initial serum antibody titers: 9 had MAT titers of ≥1/1,280; 14 had Coombs titers of ≥1/5,120; 14 had BCAP titers of ≥1/10,240; and 12 had BCAPV titers of ≥1/10,240.

No correlation was observed between the duration of disease before hospitalization and titers upon admission ($P > 0.05$), although MAT titers did show a tendency to decrease in cases with prolonged disease.

Relationship between the results of the classical serological tests and those of the BCAP test. The concordances between the MAT, the Coombs test, the BCAP test, and the BCAPV test are shown in Fig. 2. Spearman's rho coefficients were 0.982 between the BCAP and BCAPV tests ($P < 0.001$), 0.878 and

TABLE 1. Coincident pairs between BCAP titers and BCAPV titers

Reciprocal BCAPV titer	No. (%) of samples with the following reciprocal BCAP titer:													% of samples
	20	40	80	160	320	640	1,280	2,560	5,120	10,240	20,480	40,960	81,920	
20	0	1	0	0	0	0	0	0	0	0	0	0	0	0.3
40	0	23	2	0	0	0	0	0	0	0	0	0	0	7.8
80	0	1	37	1	0	0	0	0	0	0	0	0	0	12.1
160	0	0	5	40	5	0	0	0	0	0	0	0	0	15.6
320	0	0	0	3	46	6	0	0	0	0	0	0	0	17.0
640	0	0	0	0	7	42	7	0	0	0	0	0	0	17.4
1,280	0	0	0	0	0	0	23	2	3	0	0	0	0	8.7
2,560	0	0	0	0	0	0	1	14	9	0	0	0	0	7.5
5,120	0	0	0	0	0	0	0	0	16	5	0	0	0	6.5
10,240	0	0	0	0	0	0	0	0	1	12	1	0	0	4.4
20,480	0	0	0	0	0	0	0	0	0	1	3	0	0	1.2
40,960	0	0	0	0	0	0	0	0	0	0	0	3	0	0.9
81,920	0	0	0	0	0	0	0	0	0	0	0	0	1	0.3
Total	0 (0.0)	25 (7.7)	44 (13.7)	44 (13.7)	58 (18.0)	48 (14.9)	31 (9.7)	16 (5.0)	29 (9.0)	18 (5.6)	4 (1.2)	3 (0.9)	1 (0.3)	

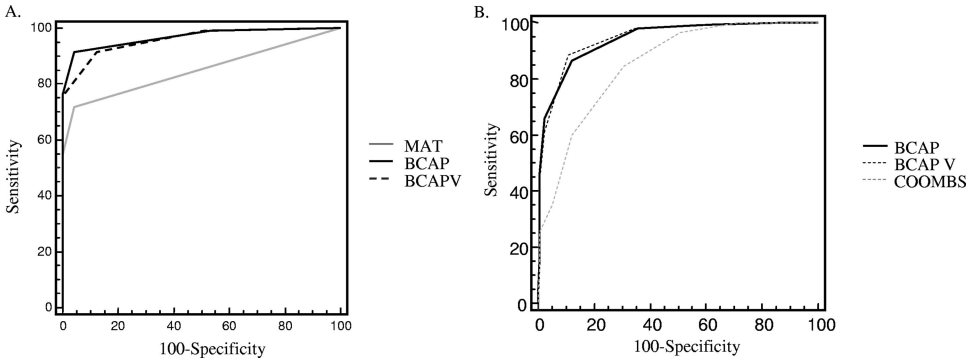

FIG. 3. (A) AUCs for the ROC curve for the BCAP test, the BCAPV test, and the MAT when the Coombs test was considered the gold standard. The AUCs for the BCAP and BCAPV tests were very similar (0.969 and 0.960) but higher than that for the MAT (0.850) ($P < 0.001$). (B) AUCs for the ROC curve for the BCAP, BCAPV, and Coombs tests when the MAT was considered the gold standard. The AUCs for the BCAP and BCAPV tests were very similar (0.942 and 0.945) but higher than that for the Coombs test (0.853) ($P < 0.001$).

0.866 between the BCAP test and the Coombs test and the MAT, respectively ($P < 0.001$), and 0.876 and 0.869 between the BCAPV test and the Coombs test and the MAT, respectively ($P < 0.001$). The correlation between the Coombs test and the MAT was 0.696 ($P < 0.001$). Multiple regression analysis indicated an independent relationship between the BCAP or BCAPV test (the dependent variables) and the Coombs test ($P < 0.005$) and the MAT ($P < 0.001$). No significant differences were observed when these correlations were evaluated separately in samples from different periods of disease evolution.

When the coincidence of pairs was evaluated for the BCAP and BCAPV tests, titers were coincident in 260 cases (81%) and noncoincident in 61 cases (19%) (weighted kappa, 0.926). For the noncoincident pairs, BCAP titers were 1 dilution higher in 39 cases and 2 dilutions higher in 3 cases, while BCAPV titers were 1 dilution higher in 19 cases (Table 1). Higher BCAP titers prevailed up to titers of ≥1/640 ($P < 0.05$).

When the Coombs test was considered the gold standard, the AUCs for the ROC curve ranged between 0.850 and 0.969, and the difference between the MAT and the BCAP or BCAPV test was statistically significant ($P < 0.001$); however, no significant difference was observed between the BCAP and BCAPV tests (difference, 0.009; $P = 0.078$). When the MAT was considered the gold standard, the AUCs for the ROC curve were similar for the BCAP (0.942) and BCAPV (0.945) tests but were lower for the Coombs test (0.853); the difference between the BCAP or BCAPV test and the Coombs test was statistically significant ($P < 0.001$) (Fig. 3).

The sensitivities and specificities of BCAP, BCAPV, and MAT titers with respect to a gold-standard Coombs titer of ≥1/160, and those of BCAP, BCAPV, and Coombs titers with respect to a gold-standard MAT titer of ≥1/80, are shown in Tables 2 and 3, respectively.

Evolution of serological results over time for 40 patients without relapse. Antibody titers in serum samples from the 40 patients without relapse decreased over time. For one patient who was in regular contact with sheep, a transitory increase of ≥2-fold in titers was detected by all tests, though he remained symptom free. Isolated and transitory increases were observed in the BCAPV and Coombs titers (one sample each) for patients who remained well.

The decreases in the BCAP and BCAPV titers were very pronounced in comparison with the Coombs titers. Thus, if the initial BCAP and BCAPV titers were two or more times higher than the Coombs titers, by the end of treatment the former were already lower. Over the following months, these three test titers decreased slowly in a similar way, although the Coombs titers showed a tendency to persist at higher levels than the BCAP and BCAPV titers.

The differences in the evolution of the BCAP and Coombs titers were further illustrated when the coincidence of pairs was analyzed: before therapy, there were 5 coincident pairs, while in 22 cases BCAP titers were higher and in 7 cases they were lower than Coombs titers. At the end of therapy or in the first month posttherapy, there were 29 coincident pairs, while

TABLE 2. Sensitivities, specificities, LR, and predictive values of BCAP, BCAPV, and MAT titers with respect to gold-standard Coombs test titers of ≥1/160

Serological test	Titer	Sensitivity (%)	Specificity (%)	LR for:		PPV[a] (%)	NPV[a] (%)
				Positive results	Negative results		
BCAP	≥160	91.6	95.9	22.4	0.09	99.2	67.1
BCAPV	≥160	91.6	87.8	7.5	0.1	97.7	65.2
MAT	≥40	71.9	95.9	17.6	0.29	99	37.9

[a] PPV, positive predictive value; NPV, negative predictive value.

TABLE 3. Sensitivities, specificities, LR, and predictive values of BCAP, BCAPV, and Coombs titers with respect to gold-standard MAT titers of ≥1/80

Serological test	Titer	Sensitivity (%)	Specificity (%)	LR for:		PPV[a] (%)	NPV[a] (%)
				Positive results	Negative results		
BCAP	≥640	86.7	87.9	7.14	0.15	86.1	88.4
BCAPV	≥640	88.7	89	8.07	0.13	87.5	90.1
Coombs	≥640	84.7	69.4	2.76	0.22	70.6	83.9

[a] PPV, positive predictive value; NPV, negative predictive value.

in 22 cases BCAP titers were higher than Coombs titers and in 23 cases they were lower. Over the following months, there were 56 coincident pairs, while 30 BCAP titers were higher and 70 were lower than Coombs titers.

When this evolution of serological results in patients without relapse was compared between the group of 26 patients without focal disease and the group of 14 patients with focal disease, a slower decrease in titers for patients with focal disease was observed by all tests. The differences between the mean $\log_{10}$ titer upon admission and that at the 12th month of follow-up were as follows: 1.13 versus 1.59 by the BCAP test, 1.08 versus 1.53 by the BACPV test, 0.92 versus 1.17 by the Coombs test, and 0.73 versus 1.32 by the MAT for patients with and without focal disease, respectively.

The time to negative results was 13 months for the MAT, 30 months for the BCAP test, 29 months for the BCAPV test, and 30 months for the Coombs test. Kaplan-Meier analysis indicated the following cumulative probabilities of persistence of serum antibody titers 12 months after therapy: 35% by the MAT, 80% by the BCAP test, 80% by the BCAPV test, and 87% by the Coombs test (Fig. 4).

Serological outcomes for eight patients with relapses. Of the nine episodes of relapse (affecting eight patients), serological results were available for eight episodes. Serological changes during relapse were detected in seven cases (88%) by the Coombs test (four cases with ≥2-dilution increases in titers and three cases with only 1-dilution increases), in five cases by the BCAP (two cases with ≥2-dilution increases and three cases with only 1-dilution increases) and BCAPV (one case with ≥2-dilution increases and four cases with only 1-dilution increases) tests, and in three cases by the MAT (one case with a ≥2-dilution increase and two cases with only 1-dilution increases) (Table 4). These changes were more evident in bacteremic relapses occurring at least 3 months after therapy (Fig. 5). For patients 1 and 2 (Table 4), clinical reactivation of spondylitis and orchitis, respectively, was observed 1 month after the end of treatment, and concomitant increases in Coombs test titers from 1/1,280 to 1/5,120 and from 1/1,280 to 1/2,560, respectively, were detected.

Persistence of high titers 12 to 18 months after therapy. Thirty-three of the 40 patients who did not relapse were able to be followed up clinically and serologically at least 12 to 18 months after therapy. Eleven of these patients had high final serum antibody titers as follows: ≥1/320 (seven cases) by the BCAP test, ≥1/320 (eight cases) by the BCAPV test, ≥1/640 (seven cases) by the Coombs test, and ≥1/80 (five cases) by the MAT. The remaining 22 patients had low final serum antibody titers. The clinical evaluation was satisfactory both for patients with high final titers and for those with low final titers. There was a good correlation between serum antibody titers upon admission and those at 12 to 18 months; thus, among 15 patients with high initial titers, 9 had high final titers, compared to only 2 of the 18 patients who did not have high initial titers ($P = 0.003$). The influence of focal disease in this persistence of high titers was less relevant.

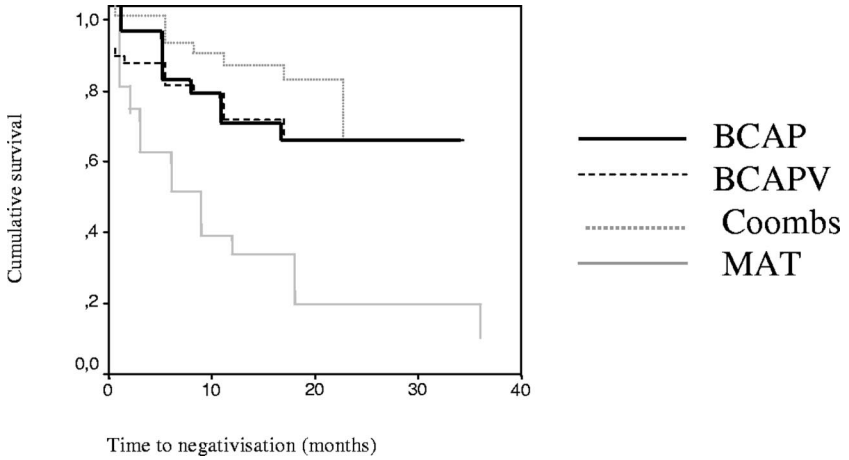

FIG. 4. Probabilities of persistence of positive titers over time for the BCAP, BCAPV, and Coombs tests and the MAT. Kaplan-Meier curves for the BCAP, BCAPV, and Coombs tests and the MAT show similar decreases in BCAP and BCAPV titers, both earlier than the decreases in Coombs titers. The MAT titers decreased even earlier than the BCAP and BCAPV titers.

TABLE 4. Antibody titers in prerelapse and postrelapse serum samples from eight cases of relapse, determined by the BCAP, BCAPV, and Coombs tests and the MAT

Case	Time of relapse (mo)	Status[a] of blood cultures at relapse	Focal disease in:		Titer[b] by the:							
			Initial episode	Relapse	BCAP test		BCAPV test		Coombs test		MAT	
					Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	1	Neg	Spondylitis	Spondylitis	2,560	2,560	2,560	2,560	**1,280**	**5,120**	320	160
2	1	Neg	Orchitis	Orchitis	10,240	10,240	5,120	5,120	1,280	2,560	640	320
3	1	Neg	Orchitis	Orchitis	640	1,280	640	1,280	640	1,280	80	80
4	1	Neg	Coxitis	Orchitis	320	640	320	640	640	1,280	40	40
5	3	Pos	Sacroiliitis	Orchitis	**640**	**10,240**	**640**	**10,240**	**320**	**2,560**	**80**	**640**
6	3	Pos	No	No	1,280	2,560	1,280	2,560	**1,280**	**5,120**	160	320
7	6	Neg	Spondylitis, arthritis, orchitis	Arthritis	5,120	2,560	5,120	2,560	10,240	5,120	160	160
8	>12	Pos	Sacroiliitis	No	**1,280**	**5,120**	640	1,280	**640**	**2,560**	80	160
Mean					1,280	2,560	**960**	**2,560**	1,280	2,560	120	160

[a] Neg, negative; Pos, positive.
[b] Pre and Post, titers for prerelapse and postrelapse serum samples, respectively. For cases in which the titer increased by ≥2 dilutions, both the prerelapse and the postrelapse titer are boldfaced; for cases in which the titer increased by only 1 dilution, both titers are underlined.

DISCUSSION

The results of our study are in accordance with those reported in previous series regarding the high sensitivity and specificity of the BCAP test in the diagnosis of human brucellosis (1, 8, 15, 17, 27, 29).

To date, the BCAP test has been considered an immunocapture-agglutination test whose ability to detect agglutinating and nonagglutinating antibodies successfully has been related mostly to the use of plates coated with antibodies against human immunoglobulins (1, 8, 15, 17, 27). However, the present study demonstrates that performing a modified but similar test using the same antigen and technique, but with plates that are not coated with immunoglobulins (the BCAPV test), yields results similar to those of the standard BCAP test. Thus, the high ability of the BCAP test to detect anti-*Brucella* antibodies is not related specifically to the mechanism of immunocapture but rather is probably due to the characteristics of the antigen and the acid pH conditions of the test (24). This is a very important finding, not only because it improves our understanding of the mechanisms involved in this test but also because the test may be simplified and its cost reduced.

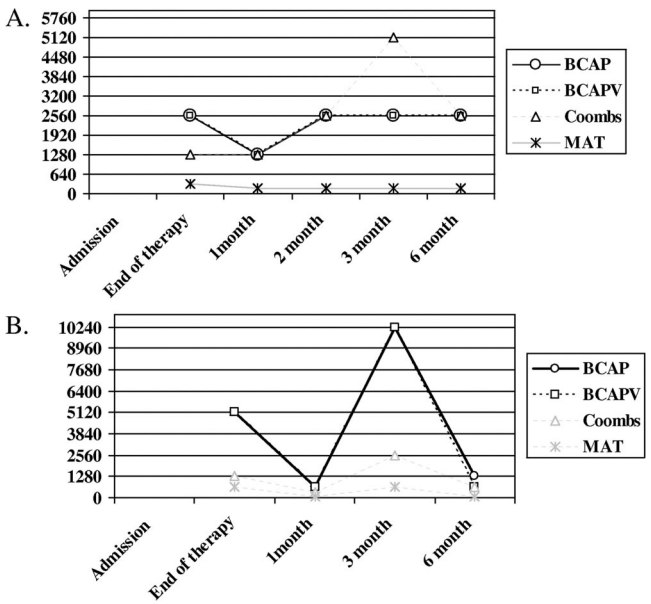

FIG. 5. Graphs of titers for two different patients with relapses 3 months after the end of therapy. (A) Case 6 from Table 4. Note that the Coombs titers increased, while the BCAP, BCAPV, and MAT titers remained unchanged. (B) Case 5 from Table 4. Note that the BCAP, BCAPV, and Coombs titers increased, while the MAT titers remained unchanged. Note also that the increases in the BCAP and BCAPV titers were much more pronounced than that in the Coombs titer. In both panels, the BCAP line is hidden behind the BCAPV line.

In this study, the BCAP test had a sensitivity of 100% for the diagnosis of initial disease, given that all patients had titers of ≥1/320 at admission (median, 1/2,560), which often were several times above the suggested diagnostic threshold of 1/160 to 1/320.

When the evolution of serological titers was evaluated over time for patients with good clinical outcomes, we observed, as reported by others (8, 15, 17, 27), a more pronounced and rapid decrease in BCAP titers than in Coombs titers, which was already evident in samples at the end of therapy or soon after treatment. The high sensitivity of the BCAP test, coupled with this rapid decrease in its titers with treatment, suggests that the test detects mainly high-affinity antibodies. Furthermore, these findings indicate that the BCAP test could be a better marker of infection activity and therefore a promising substitute for the Coombs test in the follow-up of patients with brucellosis. However, some additional points should be taken into consideration before such a conclusion is drawn.

After the rapid decrease in BCAP titers detected during the first few months, a slower reduction occurs. Thus, BCAP/BCAPV and Coombs titers remained positive at 12 months posttherapy for 80% and 87% of our patients, respectively; the decrease in titers was particularly slow for patients with focal disease. It should be noted that about 20 to 25% of patients with good clinical outcomes had BCAP titers of ≥1/320 in samples obtained 12 to 18 months after therapy, a finding that was related mainly to the detection of very high titers by this test at the initial disease point, as was observed for the Coombs

test. Overall, the BCAP test seems to be more specific than the Coombs test as a marker of disease activity; the detection of a titer of <1/160 makes present or future activity of the disease very unlikely.

At the time of relapse, a serological increase in titers should be observed in order to confirm this diagnosis (30). We previously reported increases in the levels of IgG antibodies in more than 80% of relapsing patients, as detected by an IgG enzyme-linked immunosorbent assay and the Coombs test (2, 22), and it has been suggested that the BCAP test may have a similar sensitivity (8, 17, 29). However, in the present study, while all patients had BCAP titers of ≥1/320 during relapse, serological movement was observed only in 62% of cases by the BCAP test compared to 87% of cases by the Coombs test, and these were changes of ≥2 dilutions in 25% and 50% of cases, respectively. The fact that an increase in these titers was detected only in exceptional cases in the group of patients without relapse confers a high specific value on the increase in the Coombs titer as a marker of relapse. Thus, the sensitivity of the Coombs test could be higher than that of the BCAP test, because the Coombs test detects agglutinating and nonagglutinating antibodies, including those with high and low affinities (4, 11, 21). Taken together, these findings seem to indicate that a movement of low-affinity antibodies alone may be detected in some patients with active disease; in this regard, we recently reported similar findings in the setting of late hepatosplenic reactivated brucellosis (4, 11).

We conclude that the BCAP test is a very sensitive, specific, and simple test for the routine diagnosis and management of human brucellosis. However, its efficacy is not due to an immune capture effect, because similar results could be obtained with the same technique but using plates that were not coated with antibodies against immunoglobulins; rather, it probably depends on the characteristics of the antigen used and the acid pH conditions of the test. This is relevant in terms of cost reduction, because it means that the test can be used with plates that are not coated with antibodies against immunoglobulins. With regard to detecting mainly high-affinity antibodies, the BCAP test is more specific than the Coombs test. However, if the use of the BCAP test as a possible substitute for the Coombs test is considered, it should be taken into account that in some cases of relapse and chronic forms of the disease, slight changes in low-affinity antibodies alone are observed, and these are better detected by the Coombs test.

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